Remarks

Claims 9, 18, and 19 were pending in the subject application. By this Amendment, claims 9, 18, and 19 have been canceled, and new claims 23-40 have been added. The undersigned avers that no new matter is introduced by this amendment. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 23-40 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Claims 9, 18, and 19 are rejected under 35 U.S.C. §112, first paragraph, as lacking sufficient written description and as non-enabled. The applicants respectfully submit that the subject specification provides sufficient written description and enables claims 9, 18, and 19. However, by this Amendment, the applicants have canceled claims 9, 18, and 19 and added new claims 23-40.

The Office Action indicates that the specification fails to enable a method for screening potential drugs or for the detection of virulence by utilizing a peptide encoded by a recited operon. New claims 23-40 recite a method for screening a potential antimicrobial drug by contacting a peptide with the potential antimicrobial drug and determining whether the potential antimicrobial drug inhibits the ability of the peptide to translocate a protein from the bacterial cytoplasm to the periplasm. Support for the claimed subject matter can be found, for example, at page 2, lines 15-17, page 5, lines 5-7, and page 16, lines 6-7, of the specification, and the claims as originally filed. Thus, new claims 23-40 recite affirmative steps for carrying out the method of the subject invention. In view of the guidance provided in the subject specification, and the level of skill of those in the art, one of ordinary skill in the art could carry out the method of claims 23-40 without resort to undue experimentation. Thus, the applicants respectfully submit that claims 23-40 are fully enabled by the subject specification.

Claim 23 further recites that the peptide is obtainable from *E. coli* K1 and is encoded by an operon comprising a gene selected from the group consisting of *tatA*, *tatB*, *tatC*, and *tatE*, or a homologue or functional fragment of any of the foregoing, wherein the homologue is obtainable from a Gram-negative bacterium and has at least 30% homology at the nucleotide or amino acid

level. Support for the claimed subject matter can be found, for example, at page 1, lines 20-32, page 2, lines 1-7, and page 5, lines 8-10, of the subject specification.

New claims 23-26, 28, 30-33, 35, 37, and 38 recite certain homologies (*e.g.*, at least 30%, at least 70%, at least 80%, at least 90%) and that the homologue is obtainable from a Gram-negative bacterium. Support for the claimed subject matter can be found, for example, at page 5, lines 5-10, of the subject specification. The Office Action indicates that the specification does not teach how to obtain a homologue or functional fragment. The claimed method recites that the homologues must have the stated level of homology and have the function of the Tat translocase, *i.e.*, the ability to translocate a protein from the bacterial cytoplasm to the periplasm. The applicants respectfully submit that there is sufficient information within the subject specification to obtain homologues from other Gram-negative bacteria, based on existing knowledge of Tat proteins and the sequence information provided in the specification. Methods for obtaining homologues and functional fragments of nucleotide sequences and amino acid sequences are well known in the art. As indicated at page 4, lines 24-28, of the specification, sequence homologies can be established by searching existing databases, such as the EMBL Nucleotide Sequence Database Collaboration or GenBank of the National Center for Biotechnology Information (NCBI). With the benefit of the subject specification, fragments of the *tat* nucleotide sequences and peptides encoded by those sequences that retain Tat activity can be obtained by one of ordinary skill in the art. The skilled artisan can determine suitable fragments that retain the translocation properties of the native molecule without resort to undue experimentation. Since prior to 1984, it has been well-known that *Bal*31 exonuclease can be conveniently used for time-controlled limited digestion of DNA. See for example, Maniatis, *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY, pages 135-139. Given any known DNA sequence, the skilled artisan, by using *Bal*31 exonuclease, could easily have removed nucleotides from either or both ends of the DNA molecule to systematically, routinely, and certainly generate a wide spectrum of DNA fragments from all along the length of the molecule in one afternoon; and then introduce them into host cells.

Submitted herewith for the Examiner's consideration are the Ochsner *et al.* and Lee *et al.* publications. The Ochsner *et al.* publication states that the Tat apparatus is well conserved among important bacterial pathogens, confirming what is taught in the subject specification. At page 8317, column 2, lines 5-8, Ochsner *et al.* indicate that "[a] brief screening of microbial genome sequences indicates that the TAT apparatus is well conserved among bacterial pathogens, including Mycobacterium tuberculosis, Staphylococcus aureus, and Helicobacter pylori." Furthermore, the Lee *et al.* publication shows that fragments of the proteins that retain Tat activity can be obtained by truncation experiments. As indicated in the abstract of the Lee *et al.* publication, C-terminal truncation analysis of the TatA protein in *E. coli* revealed that a TatA protein shorted by 40 amino acids retains protein translocation ability. The *E. coli tatA* gene encodes a polypeptide of 89 amino acids (page 5873, column 2, of the Lee *et al.* publication). Furthermore, similar truncation analysis showed that the final 30 amino acid residues of the TatB polypeptide are not necessary to retain translocation function, and significant translocation function was maintained even upon removal of 70 amino acid residues (see abstract and Figure 2 of the Lee *et al.* publication). As described in the Materials and Methods section of the Lee *et al.* publication at page 5872-5873, construction of plasmids carrying truncations of the *tatA* and *tatB* genes, expression of truncated forms of *tatA* and *tatB*, and subsequent translocation activity measurements were carried out using methods well known to those of ordinary skill in the art. Thus, the applicants submit that the subject specification contains sufficient disclosure to convey to one of ordinary skill in the art that the applicant had possession of the concept of what is claimed, which is all that is necessary to satisfy the written description requirement of 35 U.S.C. §112, first paragraph.

At page 5, the Office Action indicates that the subject specification does not provide functional or structural characterization of the full-length open reading frame of the peptide of SEQ ID NO:12. However, the nucleotide coding sequence for the peptide of SEQ ID NO:12 appears as the second open reading frame in SEQ ID NO:10 (nucleotides 1280-1792) within the Sequence List, as indicated in Example 5, at page 9, lines 2-13, of the subject specification.

The applicants respectfully submit that the subject specification provides sufficient written description and enables claims 23-40. Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 9, 18, and 19 are rejected under 35 U.S.C. §112, first paragraph, as non-enabled by the subject specification. The Office Action indicates that the specification does not enable a peptide having a sequence that is "homologous to a homologue at the amino acid or nucleotide level". As indicated above, the applicants have cancelled claims 9, 18, and 19, and added new claims 23-40, obviating this rejection.

Claims 23-40 recite that the homologues must have the stated level of homology and have the function of the Tat translocase, *i.e.*, the ability to translocate a protein from the bacterial cytoplasm to the periplasm. Claims 23-26, 28, 30-33, 35, 37, and 38 recite various levels of sequence homology (*e.g.*, at least 30%, at least 70%, at least 80%, at least 90%). The applicants respectfully submit that there is sufficient information within the subject specification to obtain the recited homologues from other Gram-negative bacteria, based on existing knowledge of Tat proteins, and the sequence information provided in the specification. Methods for obtaining homologues and functional fragments of nucleotide sequences and amino acid sequences are well known in the art. As indicated at page 4, lines 24-28, of the specification, sequence homologies can be established by searching existing databases. As noted above, the Ochsner *et al.* publication states that the Tat apparatus is well conserved among important bacterial pathogens. Furthermore, as indicated at page 9, lines 3-5, of the subject specification, the nucleotide sequence of SEQ ID NO:10 has homology with the *tatABCD* operon of *E. coli* K12, for which the EMBL and GenBank accession numbers are provided. Submitted herewith for the Examiner's convenience are the sequences for accession numbers AJ005830, AE000459, and AE000167, which are cited at page 9, lines 3-5 of the specification, showing that the complete *E. coli* K12 genome was published in 1997. Translation of the nucleotide sequence of SEQ ID NO:10 revealed amino acid sequences corresponding to TatA (SEQ ID NO:11), TatB (SEQ ID NO:12), TatC (SEQ ID NO:13), and TatD (SEQ ID NO:14). In view of the guidance provided in the subject specification, and the level of skill of those in the art, one of ordinary skill could carry out the method of claims 23-40 without resort to undue experimentation. Thus, the applicants

respectfully submit that claims 23-40 are fully enabled by the subject specification. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 9, 18, and 19 are rejected under 35 U.S.C. §112, first paragraph, as containing new matter. As indicated above, the applicants have cancelled claims 9, 18, and 19 and added new claims 23-40, obviating this rejection.

New claims 23-40 recite a method for screening a potential antimicrobial drug by contacting a peptide with the potential antimicrobial drug and determining whether the potential antimicrobial drug inhibits the ability of the peptide to translocate a protein from the bacterial cytoplasm to the periplasm. Support for the claimed subject matter can be found, for example, at page 2, lines 15-17, page 5, lines 5-7, and page 16, lines 6-7, of the specification, and the claims as originally filed. As the Examiner is aware, it is well settled in patent law that the claim language of an amendment need not be disclosed word for word in a specification. *In re Wilder*, 222 USPQ 369, 372 (Fed. Cir. 1984) ("It is not necessary that the claimed subject matter be described identically, but the disclosure must convey to those skilled in that art that applicant had invented the subject matter later claimed.") (emphasis added). *In re Alton*, 37 USPQ 2d 1578, 1584 (Fed. Cir. 1996) ("If a person of ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate written description requirement is met."). The applicants respectfully submit that the steps recited in claims 23-40 are implicit in the disclosure of the subject specification. The subject specification teaches that the *tat* system is a *Sec*-independent export pathway that permits translocation of fully folded proteins to the periplasm through a gated pore (page 9, lines 5-9 of the specification) and that the genes and peptides disclosed therein can be used as targets for screening potential antimicrobial drugs (page 2, lines 15-17; page 5, lines 5-7). The applicants respectfully submit that the subject specification reasonably conveys to one of ordinary skill in the art that the inventors were in possession of the claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 9, 18, and 19 are rejected under 35 U.S.C. §112, second paragraph, as indefinite. As indicated above, the applicants have cancelled claims 9, 18, and 19, and added new claims 23-40. The new claims recite a method for screening a potential antimicrobial drug by contacting a peptide with the potential antimicrobial drug and determining whether the potential antimicrobial drug inhibits the ability of the peptide to translocate a protein from the bacterial cytoplasm to the periplasm, wherein the peptide is obtainable from *E. coli* K1 and is encoded by an operon comprising a gene selected from the group consisting of *tatA*, *tatB*, *tatC*, and *tatE*. Certain claims further recite that the gene is *tatB* and may be a homologue or functional fragment of the recited peptide. The claims reciting a homologue also characterize the homologue as being from a Gram-negative bacterium, as having a stated homology at the nucleotide or amino acid level, and as having the ability to translocate a protein from the bacterial cytoplasm to the periplasm. The claims reciting a functional fragment also characterize the fragment as having the ability to translocate a protein from the bacterial cytoplasm to the periplasm. The applicants note that new claims 23-40 do not recite SEQ ID NO:33. Thus, the applicants respectfully submit that claims 23-40 are not indefinite, and sufficiently convey the metes and bounds of the claimed subject matter to one of ordinary skill in the art. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

Claims 9, 18, and 19 are rejected under 35 U.S.C. §112, second paragraph, as being incomplete for omitting essential steps. The applicants respectfully submit that the claims do not omit essential steps. However, as indicated above, the applicants have cancelled claims 9, 18, and 19, rendering this rejection moot. New claims 23-40 recite a method for screening a potential antimicrobial drug by contacting a peptide with the potential antimicrobial drug and determining whether the potential antimicrobial drug inhibits the ability of the peptide to translocate a protein from the bacterial cytoplasm to the periplasm. The applicants respectfully submit that claims 23-40 recite affirmative steps for carrying out the recited objective. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

In view of the foregoing remarks and amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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Attachments: Petition and Fee for Extension of Time
Associate Power of Attorney
Ochsner *et al.* publication
Lee *et al.* publication
NCBI printouts of Accession Numbers AJ005830, AE000459, and AE000167